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Solvent influence on the antioxidant activity of pomegranate extracts

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Abstract

In this study we tried to assess the antioxidant activity of different parts of pomegranate fruit extracts (*Punica granatum*). In order to determine the antioxidant activity it was used the 2,2-diphenyl-1-picryl-hydrazyl method and were studied seeds or whole fruits of pomegranate, respectively crushed seeds. The solvent used for the extraction was ethanol-water solution of various concentrations, including distilled water.

Keywords: pomegranate fruit (*Punica granatum*), antioxidant activity, DPPH

1. Introduction

Pomegranate (*Punica granatum*), is a fruit that grows on small trees and it's widely used in various food products, juices and alcoholic beverages. It has it's origins in Iran and is cultivated since antiquity. Nowadays, it is cultivated mainly in the Mediterranean, in the Middle East and in Caucasus region. Also, it can be found in North and Tropical Africa, Central and South-East Asia, in California and Arizona [1-12].

In addition to culinary use, pomegranate has applications in traditional medicine, seeds and pomegranate juice being used for toning properties to the respiratory and cardiovascular system. Also, studies have been made for the pomegranate juice in terms of reducing the risk of developing cataracts [1-7].

From the chemical point of view, pomegranate seeds contain C and K vitamins, as well as polyphenols (such as ellagitanins) and flavonoids, which are an important source of fiber and micronutrients. It also contains punicic, palmitic, stearic, oleic and linoleic acids, and pomegranate juice contains estrone [8-10].

Pomegranate juice contains a large variety of polyphenols, among which A and B granatin, A, B, C and D punicacortein, 5-*O*-gallailpunicacortein D, punicafolin, punigluconin, punicalagin, 1-α-*O*-galloilpunicalagin, punicalin and 2-*O*-galloil-punicalin. The red color of the pomegranate juice is given by anthocyanins (delphinidin, cyanidin, pelargonidin-glycoside) and in pomegranate peels are prevailing catechins and gallocatechins (figure 1) [1-12].

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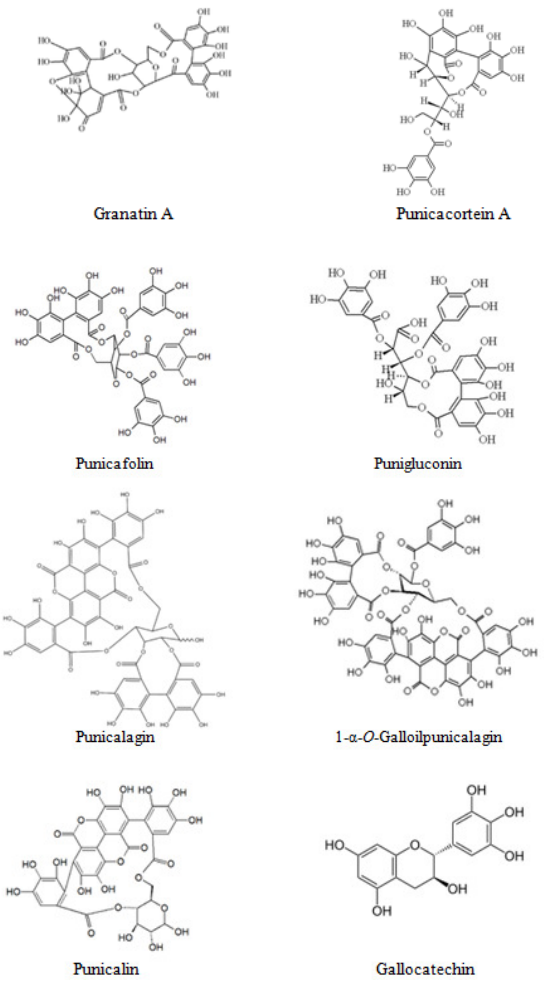

Figure 1. Main structure of polyphenolic compounds from pomegranate (http://en.wikipedia.org/wiki/Pomegranate)

The presence of these polyphenolic compounds confer to pomegranate products antioxidant properties, being demonstrated the biological effects of reducing the risk of cardiovascular diseases, including LDL (low density lipids) oxidation, reduction of systolic blood pressure, viral and bacterial infections (particularly in the plaque) [1-12].

2. Material and methods

Materials. Pomegranate fruits were purchase from the local market. The fruits were frozen, respectively the seeds, and before use they were crushed well using a mortar and pestle. For the extraction was used ethanol 96% (v/v, Chimopar, București) and distilled water to adjust the volume concentrations of ethanol to 20%, 40%, 60% and 80%. 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was purchase from Merck&Co., Inc, New Jersey and it's purity was >99%.

Obtaining of the extracts. Extracts were obtained by means of solid-liquid extraction method, using either frozen and ground whole fruits or ungound or ground seeds, respectively ethyl alcohol 96% or lower concentrations (20%, 40%, 60%, 80%) and also distilled water. There were used amounts of 10 g of the sample to 100 mL extraction solvent, the extraction being performed in the dark, at room temperature, in Erlenmeyer flasks of 250-500 mL. The flasks were stirred occasionally and maintained for 24 h (over the night), to improve the extraction (table 1). The extracts were then filtered under vacuum and subjected to spectrophotometric analysis in order to determine the antioxidant activity.

Table 1. Codes and extraction conditions for pomegranate samples

Nr	Code	Description	m_{sample} (g)	$V_{solvent}$ (mL)
1	RI	Whole pomegranate, frozen, extracted with ethanol 96%	10	100
2	SR	Pomegranate seeds, extracted with ethanol 96%	10	100
3	R0	Pomegranate extracted in water	10	100
4	R20	Pomegranate extracted in ethanol 20%	10	100
5	R40	Pomegranate extracted in ethanol 40%	10	100
6	R60	Pomegranate extracted in ethanol 60%	10	100
7	R80	Pomegranate extracted in ethanol 80%	10	100
8	R96	Pomegranate extracted in ethanol 96%	10	100

Antioxidant activity. Evaluation of antioxidant activity of pomegranate extracts was performed by spectrophotometric method using DPPH, because this relatively stable free radical presents a maximum absorbance at 517 nm, and the reaction with compounds with antioxidant activity like polyphenols lead to the production of unradicalic compounds which do not absorb at this wavelength. Therefore, monitoring the absorbance of 1 mM DPPH solution in the presence of pomegranate extract allows the evaluation of antioxidant activity, as a percentage of decrease in absorbance compared to a control sample. Absorbance monitoring was carried out for 300 seconds using a CamSpec M501 spectrophotometer, at a volume ratio of 1:1 for the DPPH solution and pomegranate extract, diluted in a ratio of 1:3 in ethanol 96%.

3. Results and Discussion

The antioxidant activity of pomegranate extracts was calculated according to the following relationship, wherein *AAO* is the amount of the antioxidant activity at time *t* (%); Abs_t is the absorbance of DPPH-pomegranate extract mixture at time *t* and $Abs_{t=0}$ is the absorbance of the same mixture at the initial time, $t = 0$.

$$AAO(\%) = 100 - \frac{Abs_t}{Abs_{t=0}} \cdot 100 = 100 \cdot \left(1 - \frac{Abs_t}{Abs_{t=0}}\right)$$

The variation of antioxidant activity of the pomegranate extracts, determined according to the method described above with the use of DPPH stable radical solution is presented in figures 2-10. There is a rapid variation of this activity until 20 s in case of ungrounded sample extracts ("RI" and "SR" samples), while crushed samples extracted with ethanol in various concentrations or with distilled water showed a slower variation of this activity, but which is significant even at 300 s (figure 10).

Table 2. Central composite design and response

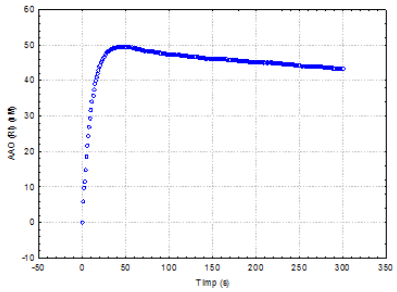

Figure 2. Variation of antioxidant activity (*AAO*) in time for "RI" sample

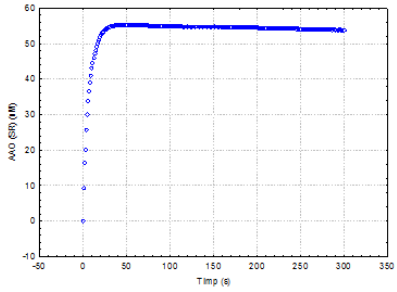

Figure 3. Variation of antioxidant activity (*AAO*) in time for "SR" sample

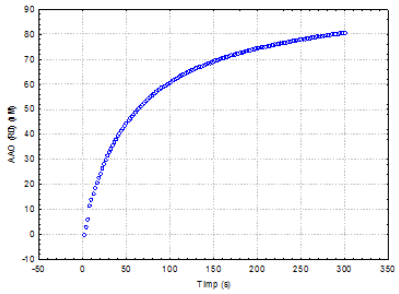

Figure 4. Variation of antioxidant activity (*AAO*) in time for "R0" sample

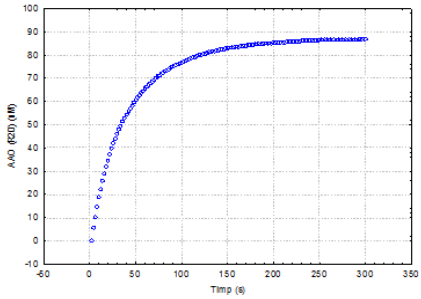

Figure 5. Variation of antioxidant activity (*AAO*) in time for "R20" sample

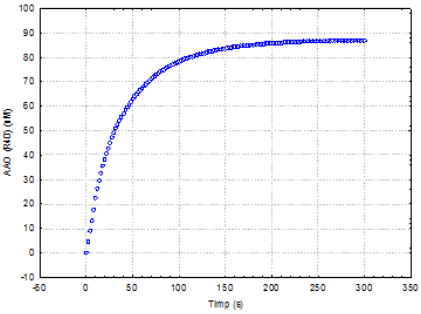

Figure 6. Variation of antioxidant activity (*AAO*) in time for "R40" sample

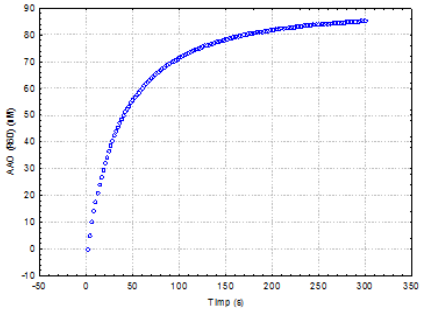

Figure 7. Variation of antioxidant activity (*AAO*) in time for "R60" sample

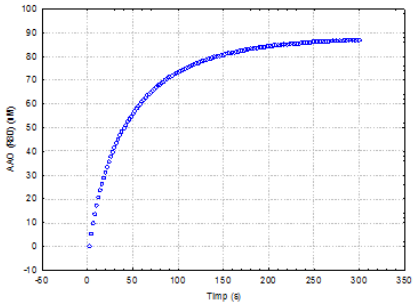

Figure 8. Variation of antioxidant activity (*AAO*) in time for "R80" sample

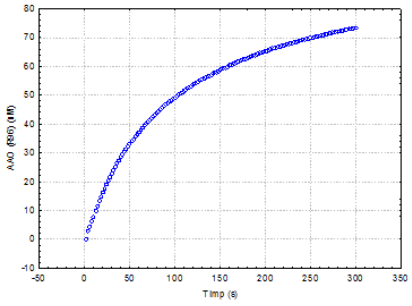

Figure 9. Variation of antioxidant activity (*AAO*) in time for "R96" sample

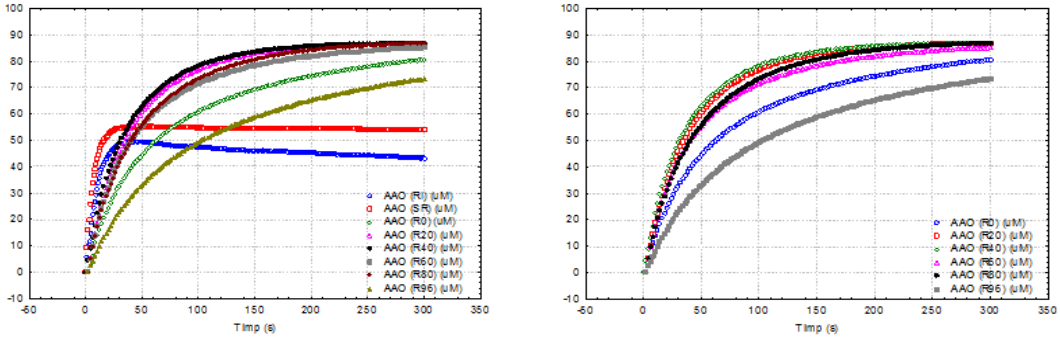

Figure 10. Variation in time of overlapped antioxidant activity (*AAO*) for all samples of pomegranate diluted extracts (above) and for those obtained by grounding and extraction with varying concentrations of ethanol

Antioxidant activity *AAO* showed values in the range 43-87%, the highest value being observed in case of well grounded pomegranate samples, extracted with ethanol 20-96% (85-87%). In this subset there is a certain variation, a maximum antioxidant activity being obtained for 40% ethanol extract (table 2). Even for the extracts obtained with distilled water, the *AAO* values were over 80% and for those obtained with concentrated ethanol only 73%. Ungrounded samples could not be extracted properly and the antioxidant activity registered a maximum value of 54% (table 2).

Much more relevant, in terms of antioxidant activity, is the reaction speed of DPPH in the presence of polyphenolic compounds from studied pomegranate extracts. To do this it was necessary to use a standard curve *Concentration = f (Absorbance)*:

$$Conc.DPPH\ (\mu M) = (Abs. - 0.025) / 10.96$$

Because DPPH shows maximum absorbance at 517 nm and reaction products with polyphenols do not absorb (or presents an insignificant absorbance) in this region, we had to resort to determining the concentration variation of DPPH in time, in the presence of studied pomegranate extracts. These variations showed a sharp decline in the first seconds of reaction (especially for the extracts obtained from ungrounded samples), followed by a gradual reduction of this decrease to near constancy in some cases (figures 11-18).

From DPPH concentration variations over time, in presence of pomegranate extracts, was found that there are three significant intervals for this variation, first between 0-20s, where the variation is maximum, second approximately between 20-80s, where the variation of the concentrations is attenuated and the last one till 300s, with the lowest variation. Therefore, it can be determine an average reaction speed of DPPH on these time intervals, in the presence of pomegranate extracts, according to the following relationship, where Δc_{DPPH} represents the

variation of DPPH concentration in the Δt time interval, in the presence of pomegranate extracts.

$$\bar{v}(\mu M/s) = -\Delta c_{DPPH} / \Delta t$$

These reaction speeds can be obtained directly from the slopes with opposite sign of the correlation lines of DPPH concentration with the reaction time, for the considered interval (figures 21 and 22). Thus, for the first period of time 0-20s, the average reaction speed was between 1-4.4 μM/s, with maximum values registered for extracts obtained from the whole pomegranate ("RI" and "SR" with $v_1 \sim 4.4$ μM/s, table 2).

Higher values were obtained using average concentrations of ethanol (2-2.8 μM/s) and lower values for extracts obtained with distilled water and with ethanol 96% (1-1.7 μM/s). For the second interval of time, the average speeds were 0.5-0.9 μM/s in case of grounded pomegranate extracts and ten times lower in case of whole samples (0.04-0.05 μM/s). Even on the last interval of time were registered significant values for these speeds of 0.05-0.14 μM/s, but only for samples obtained from crushed pomegranate seeds (table 2).

Table 2. Antioxidant activity and the reaction speed of DPPH in the presence of extracted pomegranate samples

Nr.	Code	AAO[(a)] (%)	v_1 (μM/s)[(b)]	v_2 (μM/s)[(b)]	v_3 (μM/s)[(b)]
1	RI	43.31	4.361	0.050	-
2	SR	53.86	4.355	0.042	-
3	R0	80.64	1.733	0.636	0.130
4	R20	86.79	2.062	0.651	0.057
5	R40	86.95	2.005	0.588	0.048
6	R60	85.23	2.782	0.882	0.116
7	R80	85.90	1.961	0.687	0.083
8	R96	73.35	0.971	0.496	0.138

(a) Antioxidant activity

(b) Reaction speed of DPPH, on following intervals of time: 0-20s (v_1), 20-80s (v_2), respectively on 80-300s (v_3)

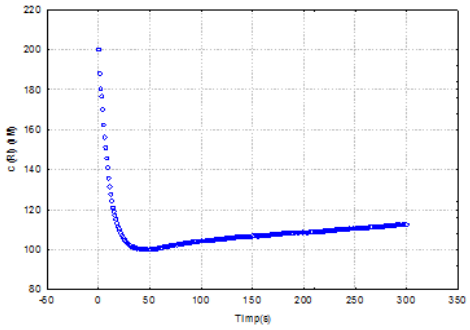

Figure 11. Variation of DPPH concentration in time, in the presence of whole pomegranate extract sample, obtained with ethanol 96%

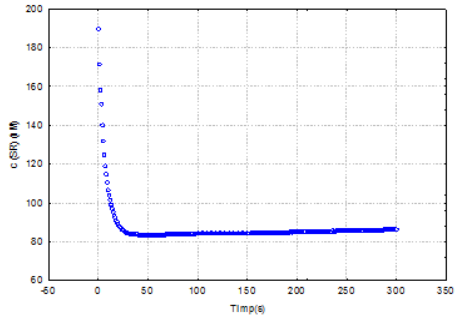

Figure 12. Variation of DPPH concentration in time, in the presence of whole pomegranate seed extract sample, obtained with ethanol 96%

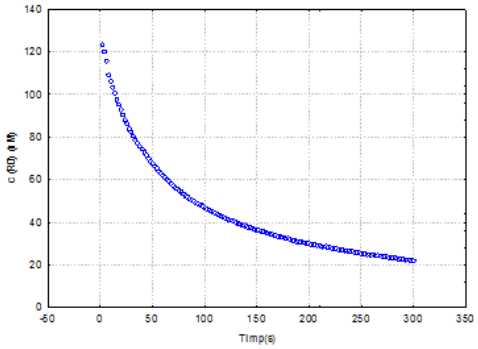

Figure 13. Variation of DPPH concentration in time, in the presence of pomegranate extract sample, obtained with distilled water

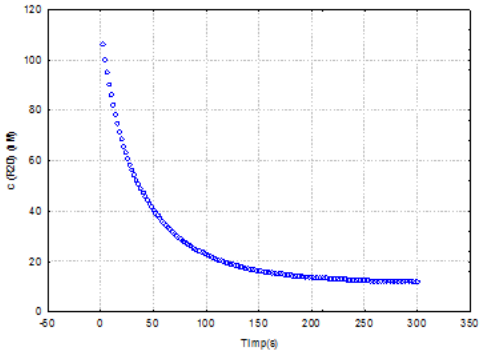

Figure 14. Variation of DPPH concentration in time, in the presence of pomegranate extract sample, obtained with ethanol 20%

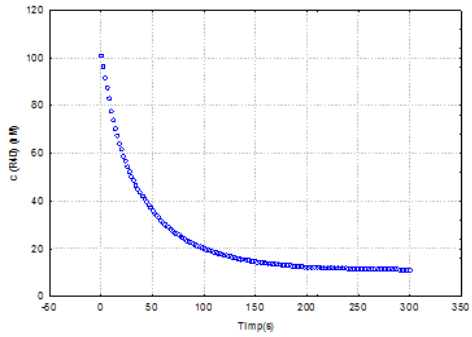

Figure 15. Variation of DPPH concentration in time, in the presence of pomegranate extract sample, obtained with ethanol 40%

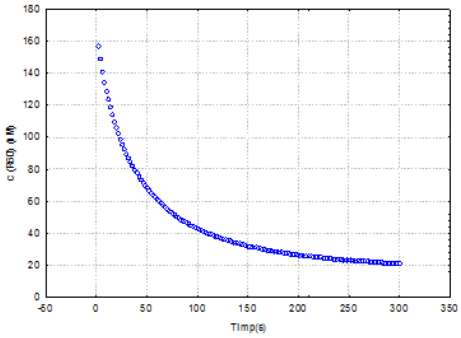

Figure 16. Variation of DPPH concentration in time, in the presence of pomegranate extract sample, obtained with ethanol 60%

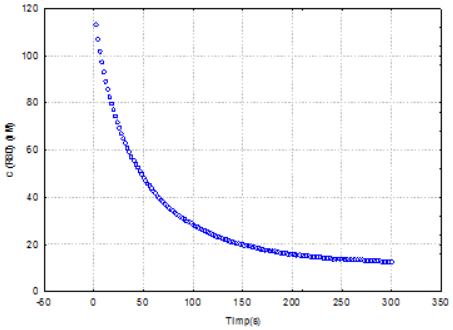

Figure 17. Variation of DPPH concentration in time, in the presence of pomegranate extract sample, obtained with ethanol 80%

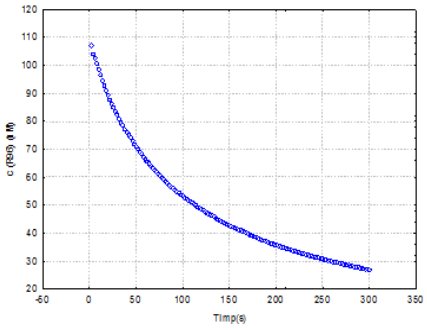

Figure 18. Variation of DPPH concentration in time, in the presence of pomegranate extract sample, obtained with ethanol 96%

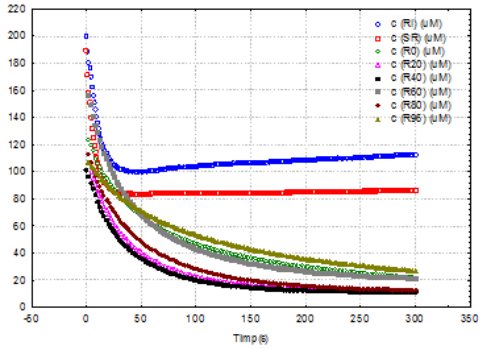

Figure 19. Overlapping of DPPH variation of concentration in time, in the presence of all samples of studied pomegranate extracts

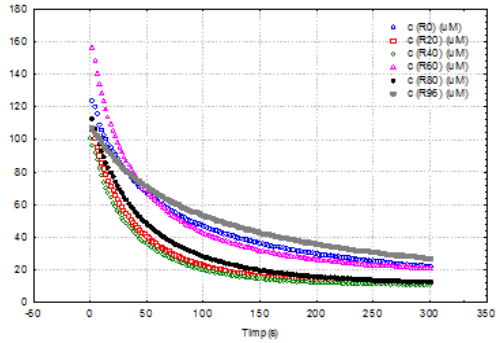

Figure 20. Overlapping of DPPH variation of concentration in time, in the presence of pomegranate extract samples extracted with ethanol 0-96%

4. Conclusions

Studies on the antioxidant activity of pomegranate extracts, obtained from different fruit parts, processed differently and using different extraction solvent mixture of ethanol-water type, lead to the following conclusions:

- All studied samples of pomegranate exhibit significant antioxidant activity, but the sample preparation is very important for an efficient extraction of antioxidant compounds;
- Most important antioxidant activity was obtained using ethanol 40% as extraction solvent for pomegranate seeds;
- Reaction speed of DPPH free radical, in presence of polyphenolic compounds from pomegranate extracts, is an important parameter for the evaluation of the antioxidant activity and for long-term effect of this kind of food products.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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